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September 17, 2007

Affidavit 1.132

I have worked as a clinical embryologist for fourteen years in the United States. I have been licensed practitioner of microsurgical fertilization (ICSI) and embryo biopsy in the UK. I have worked at Reprogenetics for eleven months and have been working to isolate disomic stem cells derived from trisomic embryos for 3 months. I am a relative novice in stem cell biology. I have read and examined Santiago Munne's patent application 20050019907 and the methods outlined in his publication. I have followed the protocols and methods outlined in his publication with some adaptations that minimize the use of animal components in the culture system. This work is preliminary and full characterization for pluripotency is not yet complete. A current cell line, derived from an embryo trisomic for chromosomes 16 and 18 and monosomy 22, from which disomic cells have been isolated, was cultured and characterized according to the following protocol.

Human embryos classified as chromosomally abnormal after Preimplantation Genetic Diagnosis (PGD) and not selected for the establishment of pregnancy were donated according to IRB approved consent (WIRB protocol 20041976) and signed by each patient. In most cases a single cell was biopsied from day 3 embryo and the nucleus analyzed for chromosome aneuploidy. When a nucleus was not observed in the first cell, a second was biopsied. All cells were analyzed for chromosomes X, Y, 13, 15, 18, 21, and 22 using FISH protocols described by Santiago Munne in the literature. Based on these results, individual embryos were selected for the study. Trisomic and monosomic embryos were continued in a culture of complex media (Global medium, IVF Online) until day 6 of development or blastocyst stage. Blastocysts with a visible inner cell mass (ICM) were selected for further culture. In this case, trophectederm biopsy was not performed for the purpose of minimizing manipulations to maximize ongoing viability. Selected blastocysts were treated with pronase or acidified tyrodes solution (IVF Online) to dissolve the zona pellucida. Zona free intact blastocysts were plated onto human foreskin fibroblasts (HFF) (ATCC-CRL-1634) previously treated with mitotic inhibitor Mitomycin-C (Sigma-Aldrich). Media used to maintain HFF feeder cells was composed of KO-DMEM 80% (Invitrogen), certified fetal bovine serum 20% (Invitrogen) L-Glutamine 2mM (Invitrogen), NEAA X1 (Invitrogen), 2-β mercaptoethanol 0.1mM (Invitrogen), Pen/Strep x1 (Invitrogen).
Blastocyts were plated on HFF feeder cell monolayers and cultured at 37°C with 5% CO_2 and observed for attachment and primary outgrowth within 48hrs. Media was then changed every 24hrs and cultured for approximately 12 days until colony formation. Putative human embryonic stem cell (hESC) colonies were maintained in the following equilibrated xeno free chemically defined medium: DMEM F-12 (Invitrogen), 1x B-27 (stock:50) (Invitrogen), 1xN2 (stock:100) (Invitrogen), L-Glutamine 200mM (2mM final

conc.) (Invitrogen), NEAA x1 (Invitrogen), 2-β Mercaptoethanol (stock 55mM)0.11mM final conc. (Invitrogen), βFGF2 20 ng/ml (Invitrogen). Morphology and growth characteristics were recorded daily.

Colonies were mechanically passaged within 14 days of plating and placed on fresh HFF feeder layers. Mechanical passaging involved the gentle teasing of the colony from the feeder layer and lifting away from the polystyrene surface with a pulled glass Pasteur pipette with polished tip, then washed through hESC medium and placed onto new HFF feeders. After 7 days another passage was carried out and colonies were dissected with 30 gauge needles under dissecting microscope and placed separately onto new feeder layers, expanding the original colony.

After the third passage (4 weeks post plating), a sample of 22 cells was fixed and analyzed by FISH for chromosomes X, Y, 13, 15, 16, 17, 18, 21, 22 as described in the literature by Santiago Munne. In this case, the specific cell line was derived from an embryo diagnosed with trisomy 16 and 18, and monosomy 22 on day 3 of development. 3/22 cells analyzed from the resulting cell line, after one month of culture, were trisomy 18, 0/22 were trisomy 16, and 14/22 were disomic for all chromosomes tested.

I have used rhodamine labeled ligand isolated to mimic TRA-1-60 antibody binding. TRA 1-60 monoclonal binds the stem cell epitope.

I have bound this ligand to cells cultured with the procedure detailed above, an approximation of the Munne protocol and in his application. (See attached micrograph) below)

This micrograph shows differential binding. There are cells binding the TRA-1-60 mimic and cells that do not bind TRA-1-60. The cells that bind are stained red indicating undifferentiated cells and those that do not bind and exhibit no staining, are differentiated.

I am also identifying cells derived from the same embryo whose parental alleles are known in order to characterize uniparental stem cells. This experiment is presently going on.

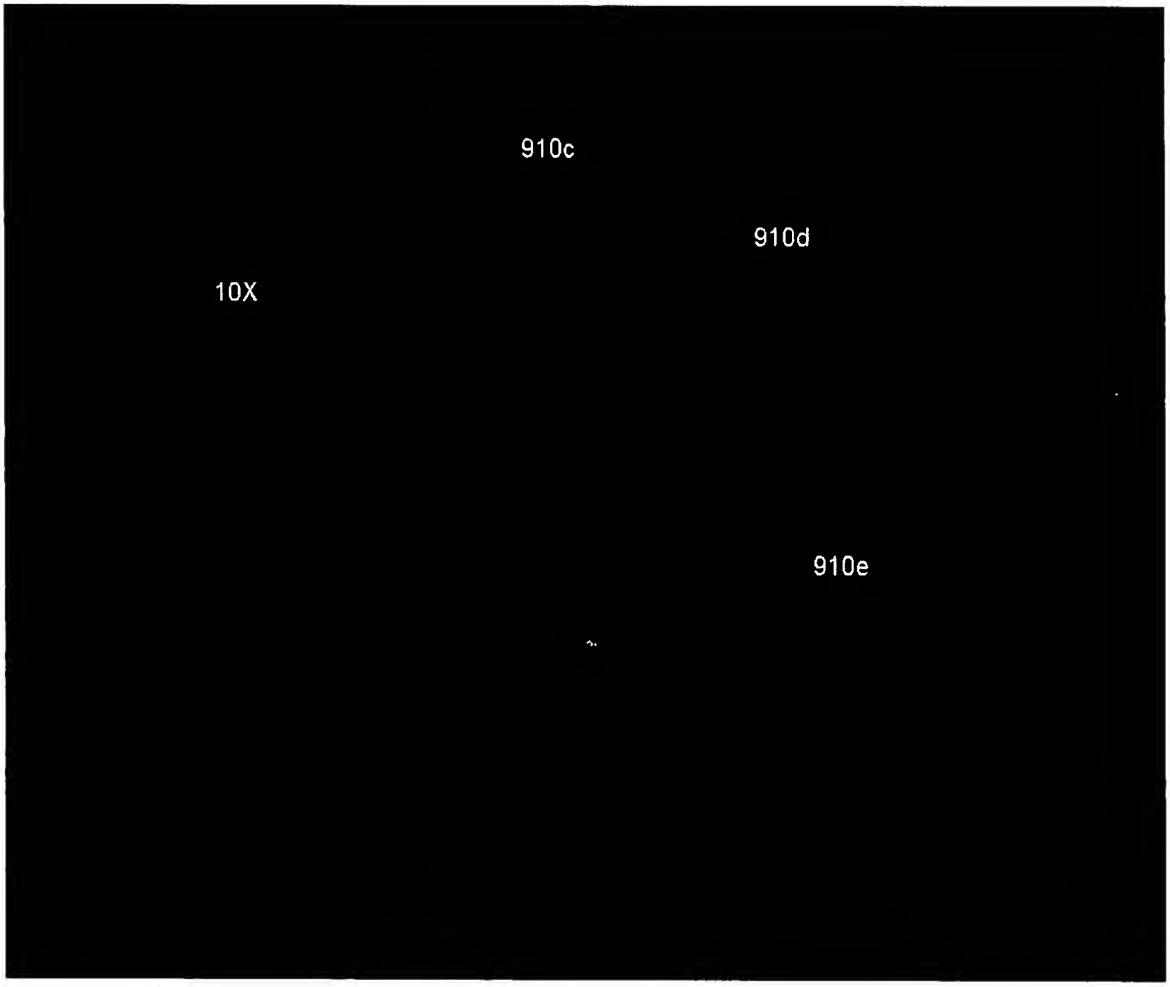
I, hereby, certify the above statements on information and belief are true.

Respectfully submitted,

Sasha Sadowy

Sworn to before me
this 19 day of
Sept .2007

CHIVA BARLATIER
NOTARY PUBLIC, STATE OF NEW YORK
QUALIFIED IN KINGS COUNTY
REG. #01BA6171169
MY COMM. EXP. JULY 23, 2011



Attached Macrograph for Sasha Sadowy affidavit